PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/54, 15/70, 9/12, 1/21, C12Q 1/48, C07K 16/40 — A1

(11) International Publication Number: WO 99/11795

(43) International Publication Date: 11 March 1999 (11.03.99)

(21) International Application Number: PCT/US98/18558

(22) International Filing Date: 4 September 1998 (04.09.98)

(30) Priority Data:
9718952.6 5 September 1997 (05.09.97) GB

(71) Applicant *(for all designated States except US)*: ICOS CORPORATION [US/US]; 22021 20th Avenue, S.E., Bothell, WA 98021 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: CARR, Antony, Michael [GB/GB]; 75 Edburton Avenue, Brighton, Sussex BN1 6EQ (GB).

(74) Agent: WILLIAMS, Joseph, A., Jr.; Marshall, O'Toole, Gerstein, Murray & Borun, 6300 Sears Tower, 233 S. Wacker Drive, Chicago, IL 60606–6402 (US).

(81) Designated States: AU, CA, CN, JP, MX, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: MAMMALIAN CHK1 EFFECTOR CELL–CYCLE CHECKPOINT PROTEIN KINASE MATERIALS AND METHODS

(57) Abstract

The present invention generally relates to genes encoding cell cycle checkpoint kinase related proteins essential to meiosis, mitosis, and DNA damage responses in cells and the respective proteins. These kinases arrest the cell cycle following DNA damage to allow DNA repair prior to mitosis, meiosis, or initiation of DNA replication. More particularly, the invention provides a novel cycle checkpoint kinase, Chkl, and polynucleotide sequences encoding Chkl. Assays for identifying modulators of Chkl are also disclosed. Modulators are useful for example, in chemotherapy and as radiation adjuvants.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

MAMMALIAN CHK1 EFFECTOR CELL-CYCLE CHECKPOINT PROTEIN KINASE MATERIALS AND METHODS

FIELD OF THE INVENTION

The present invention generally cell cycle checkpoint protein kinases which are essential to cellular DNA damage responses and coordinating cell cycle arrest. The checkpoint kinases play a role in the surveillance and response to DNA damage that occurs as a result of replication errors, DNA mismatches, radiation treatment, or chemotherapy. These checkpoint kinases are required in regulatory pathways that lead to cell cycle arrest and apoptosis following DNA damage, giving the cell notice and time to correct lesions prior to the initiation of DNA replication or chromosome separation. More particularly, the present invention relates to novel mammalian effector (Chk1) checkpoint protein kinases, polynucleotides encoding the same, and methods and materials for assaying and modulating the enzymatic activity of the kinases.

BACKGROUND

The cell cycle is structurally and functionally conserved in its basic process and mode of regulation across all eukaryotic species. The process of eukaryotic cell growth and division is the somatic (mitotic) cell cycle which consists of four phases, the G1 phase, the S phase, the G2 phase, and the M phase. The G1, S, and G2 phases are collectively referred to as interphase of the cell cycle. During the G1 (gap) phase, biosynthetic activities of the cell progress at a high rate. The S (synthesis) phase begins when DNA synthesis starts and ends when the DNA content of the nucleus of the cell has been replicated and two identical sets of chromosomes are formed. The cell then enters the G2 (gap) phase which continues until mitosis starts. In mitosis, the chromosomes pair and separate and two new nuclei form, and in cytokinesis, the cell itself splits into two daughter cells each receiving one nucleus containing one of the two sets of chromosomes. Mitosis (the M phase of the cell cycle) is immediately followed by cytokinesis. Cytokinesis terminates the M phase and marks the beginning of interphase of the next cell cycle. The sequence in which the events in the cell cycle proceed is tightly regulated such that the initiation of one cell cycle event is dependent on the completion of the prior cell

cycle event. This allows fidelity in the duplication and segregation of genetic material from one generation of somatic cells to the next.

Meiosis is the form of cell division that produces germ cells in higher eukaryotes. In contrast to mitosis, where mitotic cell division results in genetically identical cells containing two of each chromosome, meiotic cell division results in cells containing one copy of each chromosome. In addition, in meiosis homologous chromosomes pair and exchange genetic material. Meiosis consists of two stages of cell division, meiosis I and meiosis II. In meiosis I, maternal and paternal chromosomes duplicate and homologous chromosomes pair together (synapsis). The cell then undergoes division in which homologous pairs of duplicate chromosomes separate and enter individual cells resulting in two diploid daughter cells. The daughter cells then enter meiosis II. In meiosis II, the chromosomes align without further replication and sister chromatids separate, as in mitosis, to produce haploid cells. The sequence of events in both meiosis I and meiosis II are interphase, prophase, metaphase, anaphase, and telophase.

The first stage of meiosis I is interphase I in which each chromosome is replicated. The two copies of the replicated chromosome are called sister chromatids. Five sequential stages then define the first meiotic prophase. During leptotene, the newly replicated sister chromatids are in close apposition so that they may associate and undergo recombination. During zygotene, a proteinaceous structure termed the synaptonemal complex forms between maternal sister chromatids and paternal sister chromatids resulting in a bivalent (four chromatids). During pachytene, recombination between two sister chromatids (i.e. exchange of genetic material between maternal and paternal chromosomes) begins. The next stage, diplotene, is marked by the disassembly of the protein axes and the two sister chromatids begin separating. Diakinesis, the final stage, is characterized by detachment of the chromosomes from the nuclear envelope and each bivalent is clearly seen to contain four separate chromatids, with each pair of sister chromatids linked at their centromeres. Thus, early in meiosis during the "reduction division" process, sister chromatids pair and undergo reciprocal recombination at some regions. Programmed DNA strand breaks initiate recombination. [Cao et al., Cell,

88:375-384 (1997)]. The changes observed during the first meiotic prophase facilitates the genetic reassortment that assures genetic viability.

The process of monitoring genome integrity and preventing cell cycle progress in the event of DNA damage has been described as "cell cycle checkpoint" [Hartwell and Weinert Science, 246:629-634 (1989); Weinert et al., Genes and Dev., 8:652 (1994)]. Cell cycle checkpoints consist of signal transduction cascades which couple DNA damage detection to cell cycle progression. In meiosis, cell cycle checkpoints control programmed DNA breaks, ensuring the proper segregation of a complete haploid set of chromosomes to each gamete.

Failure of cell cycle checkpoints predisposes individuals to or directly causes many disease states such as cancer, ataxia telangiectasia, embryo abnormalities, and various immunological defects associated with aberrant B and T cell development. The latter are associated with pathological states such as lupus, arthritis and autoimmune diseases. Intense research efforts have therefore focused on identifying cell cycle checkpoints and the proteins essential for the function of the checkpoints.

It has been reported that cell cycle checkpoints comprise at least three distinct classes of polypeptides which act sequentially in response to cell cycle signals or defects in chromosomal mechanisms. [Carr, A.M., Science, 271:314-315 (1996)]. The first class is a family of proteins which detect or sense DNA damage or abnormalities in the cell cycle. These sensors include Atm and Atr [Keegan et al., Genes and Devel., 10:2423-2437 (1996)]. The second class of polypeptides amplify and transmit the signal detected by the detector and is exemplified by Rad53 [Alen et al. (1994) Genes Dev. 8:2416-2488]. Finally, the cell cycle checkpoint effects a cellular response, e.g. arrest of mitosis/meiosis, apoptosis through cell cycle effectors.

Genetic analysis in the yeasts Schizosaccharomyces pombe and Saccharomyces cerevisiae has identified a number of checkpoint genes important for mitotic arrest and DNA repair responses to IR. For a review, see Carr and Hoekstra, Trends in Cell Biology, 5: 32-40 (1995). One such gene, identified in yeasts, is required for a DNA damage checkpoint which arrests mitosis at the G2 phase, as well as a related checkpoint which monitors the completion of DNA synthesis and arrests the cell cycle at the S phase. The gene is named rad3 in S. pombe [Seaton et al., Gene, 119: 83-89 (1992)

Bentley et al., (1996) EM BO J. 15: 6641-6651], MEC1/ESR1 in S. cerevisiae [Kato and Ogawa, Nuc. Acids. Res., 22(15): 3104-3112 (1994)], but is hereinafter referred to as rad3. Cells having mutations in rad3 fail to either sense or appropriately respond to DNA damage and subsequently lose viability more rapidly than wild type cells after exposure to clastogenic agents or events (e.g., IR, DNA damaging agents, and mutations affecting chromosomal integrity). See Weinert et al., GENES & DEVELOPMENT, 8: 652-665 (1994) and Al-Khodairy et al., EMBO J., 11(4): 1343-1350 (1992). Rad3 thus appears to be a checkpoint detector of DNA damage (Carr, 1996). In addition rad3 appears to function in vivo as a multimer. [Bentley et al., 1996].

The product of the rad3 gene is an approximately 270 kD protein that is a member of a growing family of high molecular weight mammalian checkpoint kinases. See Hunter, Cell, 83: 1-4 (1995) for a discussion of this family of kinases. This family includes ecl1 (S. cerevisiae) mei-41 (Drosophila melanogaster), tor1 (S. cerevisiae), tor2 (S. cerevisiae), Frap (Human), tel1 (S. cerevisiae), DNA-Pk (Human) Atr (human) and Atm (human). These proteins have been identified as members of a family based on sequence homology and complementation studies.

The human homolog of rad3, Atr (Ataxia Telangiectasia and rad3 related) was identified in Bently et al., EMBO J., 15:6641-6651 (1996). Bently et al., showed that recombinant Atr can heteromultimerize with rad3 when expressed in S. pombe. In addition, recombinant Atr expression complemented S. cerevisiae mec1 mutants.

The primary structures of the catalytic domains found in members of this kinase family are closely related to well characterized phosphatidylinositol kinases. This structural relationship initially suggested that these mammalian checkpoint kinases might be capable of phosphorylating lipids. However, when the substrate specificity of the mammalian checkpoint kinases is examined, these enzymes appear to function as protein kinases and have yet to be demonstrated to phosphorylate phosphatidylinositides.

Atm (Ataxia Telangiectasia Mutated), another member of this family was identified through the analysis of the human disease syndrome ataxia-telangiectasia (AT) [Savitsky et al., Science, 268:1749-1753 (1995) and Savitsky et al., Human Molecular Genetics, 4(11):2025-2032 (1995)]. Patients with AT exhibit a diverse set of clinical symptoms, including predisposition to a variety of tumor types. Fibroblasts from AT

patients are radiosensitive and fail to undergo mitotic arrest following treatment with IR. Mutant mice lacking Atm show gonadal atrophies, meiotic abnormalities and severe chromosome fragmentation [Ashley et al., Proc. Nat. Acad. Sci. USA, 93:13084 (1996)]. This is reminiscent of the S. pombe stains with rad3 defects where cells fail to sense or respond appropriately to DNA damage.

This family of kinases thus appear to function as detectors for defects of various cell-cycle transitions [Carr, 1996].

Recently it was shown that the mammalian Atm and Atr proteins associate with chromosomes during pachynema of meiotic prophase and may monitor strand disruptions that occur during meiotic chromosome synapsis and recombination. Localization of Atm and Atr kinases shows complementary patterns of foci during zygonema and pachynema, commensurate with different roles in monitoring the DNA structure during meiotic recombination [Keegan et al., Genes Dev., 10:2423 (1996)].

Checkpoint proteins clearly play a role in signal transduction cascades. The detectors Atm and Atr are protein kinases that comprise a early step in the signal transduction cascade. It is thought that signally is amplified by protein kinases such as Rad53 which acts to transduce a signal from the detectors to the effectors.

To date, p53 and S. pombe Chk1 and wee1 have been identified as effector checkpoint proteins.

The S. pombe Chk1 gene was isolated based on its genetic interaction with the cdc2.r4 allele [Al-Khodairy et al., Mol. Biol. Cell, 5:147-160 (1994)], and by complementation of the radiation sensitivity of a rad27 mutant. cdc2 encodes a protein kinase subunit that associates with cyclins to form active protein kinase complexes that induce passage through mitosis. [Broek et al., Nature, 349:388-393 (1991]. S. pombe Chk1 appears to effect the mitotic arrest following DNA damage and S. pombe Chk1 deletion mutants fail to undergo cell-cycle arrest after irradiation [Walworth et al. , Nature, 363:368 (1993), Al-Khodairy et al., Mol. Biol. Cell., 5:147-160 (1994), Carr, A.M., Semin. Cell Biol., 6:65-72 (1995)]. However, Chk1 is not required for the other checkpoint protein-mediated DNA cellular responses to blocks to DNA replication. Walworth and Bernards Science, 271:353-356 (1996) demonstrated that in vivo activity of Chk1 is regulated by phosphorylation of Chk1. By examining the phosphorylation

status of Chk1 in various S. pombe strains harboring mutations in checkpoint genes, Walworth and Bernards, Science, 271:353-356 (1996) demonstrated that Chk1 acts downstream of these checkpoint proteins. Chk1 phosphorylation was abolished or greatly diminished in S. pombe rad 1, rad 3, rad 9, rad 17, and rad26 mutants.

In addition, S. pombe Chk1 appears to function during the G1 and G2 phases of mitosis. Carr et al., Curr. Biol., 5:1179-1190 (1995) demonstrated that Chk1 deficient cells failed to enter the S phase indicating that Chk1 is a G1 checkpoint kinase. It was demonstrated in O'Connell et al., EMBO Journal, 16:545-554 (1997) that Chk1 phosphorylated weel, a checkpoint kinase involved in G2 cell cycle arrest.

The Drosophila homolog of S. pombe Chk1 was identified as "Grp" in Sibon et al., Nature, 388:93-97 (1997). Grp is required for cell cycle control at the mid-blastula transition (MBT) in which the maternal component of the DNA-replication machinery slows DNA synthesis and induces a checkpoint-dependent delay in cell cycle progression during embryogenesis.

The C. elegans homolog of Chk1 was first reported as an EST in Genbank Accession No. U44902. The S. cerevisiae homolog of Chk1 was identified as a probable ser/thr protein kinase in Genbank Accession No. 585344.

To date, there has been no identification of a mammalian effector checkpoint (Chk1) protein kinase. There thus exists a need in the art for identification of the mammalian effector proteins that are involved in the cell cycle checkpoints in order to develop therapies for the human disease states associated with defective cell cycle checkpoints and for the isolation of polynucleotides encoding those proteins which in themselves may be useful as therapeutics or which would enable the development of therapeutically useful modulators of the proteins encoded by the polynucleotides.

SUMMARY OF THE INVENTION

The present invention provides novel human mammalian effector cell cycle checkpoint, Chk1, kinases and polynucleotides encoding the same.

In one of its aspects, the present invention provides purified and isolated polynucleotides (e.g., DNAs and RNAs, both coding and non-coding strands thereof) encoding the human and mouse effector cell cycle checkpoint kinase and polynucleotides

encoding other mammalian checkpoint kinases that exhibit 50% or greater amino acid identity to the polynucleotide region encoding the human Chk1 kinase domain (amino acids 14 to 264 of SEQ ID NO.: 2). Preferably, the polynucleotides encode a checkpoint kinase that exhibits 70% or greater amino acid identity to amino acids 14 to 264 of SEQ ID NO.: 2. Even more preferably, the polynucleotides encode a checkpoint kinase that exhibits 90% or greater amino acid identity to amino acids 14 to 264 of SEQ ID NO.: 2. Polynucleotides contemplated by the invention include genomic DNAs, RNAs, cDNAs and wholly or partially chemically synthesized DNAs. Preferred polynucleotides of the invention comprise the human Chk1 DNA sequence set out in SEQ ID NO.: 1, the mouse Chk1 DNA sequence set out in SEQ ID NO.: 3, and DNA sequences which hybridize to the noncoding strands thereof under stringent conditions or which would hybridize but for the redundancy of the genetic code. Exemplary stringent hybridization conditions are as follows: hybridization at 65° C in 3X SSC, 20 mM $NaPO_4$ pH 6.8 and washing at 65° C in 0.2X SSC. It is understood by those of skill in the art that variation in these conditions occurs based on the length and GC nucleotide base content of the sequences to be hybridized. Formulas standard in the art are appropriate for determining exact hybridization conditions. See Sambrook et al., 9.47-9.51 in Molecular Cloning, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). A polynucleotide vector encoding human Chk1 (plasmid pGEMT-Chk1hu) was deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852 on date August 27, 1997 under Accession No. ATCC98520.

The DNA sequence information provided by the present invention makes possible the identification and isolation of DNAs encoding mammalian checkpoint related molecules by well-known techniques such as DNA/DNA hybridization as described above and polymerase chain reaction (PCR) cloning. As one series of examples, knowledge of the sequence of a cDNA encoding a mammalian Chk1 of the invention makes possible the isolation by DNA/DNA hybridization of genomic DNA sequences encoding the kinase and expression control regulatory sequences such as promoters, operators and the like. Similarly, knowledge of a partial cDNA sequence makes isolation of a complete cDNA possible. DNA/DNA hybridization procedures carried out with DNA sequences of the invention under stringent conditions are likewise expected to allow the isolation of DNAs

encoding allelic variants of the kinase non-human species enzymes homologous to the mammalian Chk1 kinase and other structurally related proteins sharing one or more of the enzymatic activities, or abilities to interact with members or regulators, of the cell cycle checkpoint pathway in which mammalian Chk1 participates. Polynucleotides of the invention when detectably labeled are also useful in hybridization assays to detect the capacity of mammalian cells to synthesize kinases of the invention. The DNA sequence information provided by the present invention also makes possible the development, by homologous recombination or "knockout" strategies [see, Capecchi, Science, 244: 1288-1292 (1989)], of rodents that fail to express a functional kinase or that express a variant thereof. Such rodents and their cells are useful as models for studying the activities of mouse and kinase modulators in vivo. Polynucleotides of the invention may also be the basis for diagnostic methods useful for identifying a genetic alteration(s) in the mammalian Chk1 gene locus that underlies a disease state or states. Also made available by the invention are anti-sense polynucleotides relevant to regulating expression of Chk1 by those cells which ordinarily express the same.

For example, primers designed from the Chk1 cDNA are useful for reverse transcriptase PCR analysis of mRNA samples from tumor cells to detect the presence or absence of Chk1 mRNA. Further, sequence information from the Chk1 genomic clone can be used for single stranded conformational polymorphism (SSCP) analysis of genomic DNA prepared from tumor cells to detect alterations or mutations of the Chk1 gene. Likewise, the Chk1 cDNA and/or the Chk1 genomic clone can be used in fluorescence in situ hybridization (FISH) analysis to detect alterations in the Chk1 gene.

The invention also provides autonomously replicating recombinant constructions such as plasmid and viral DNA vectors incorporating polynucleotides of the invention, especially vectors in which the polynucleotides are functionally linked to an endogenous or heterologous expression control DNA sequence and a transcription terminator.

According to another aspect of the invention, host cells, especially unicellular host cells such as procaryotic and eukaryotic cells, are stably transformed or transfected with DNAs of the invention in a manner allowing expression of a mammalian Chk1 kinase therein. Host cells of the invention are conspicuously useful in methods for

the large scale production of Chk1 wherein the cells are grown in a suitable culture medium and the desired enzymes are isolated from the cells or from the medium in which the cells are grown.

Chk1 products having part or all of the amino acid sequence set out in SEQ ID NO.: 2 or SEQ ID NO.: 4 are contemplated. Use of mammalian host cells is expected to provide for such post-translational modifications (e.g., myristoylation, glycosylation, truncation, lipidation and tyrosine, serine or threonine phosphorylation) as may be needed to confer optimal biological activity on recombinant expression products of the invention. The enzyme products of the invention may be full length polypeptides, fragments or variants. Variants comprise Chk1 products wherein one or more of the specified (i.e., naturally encoded) amino acids is deleted or replaced or wherein one or more nonspecified amino acids are added: (1) without loss of the protein kinase activity specific to Chk1; or (2) with disablement of the protein kinase activity specific to Chk1; or (3) with disablement of the ability to interact with members or regulators of the cell cycle checkpoint pathway. Substrates of Chk1 and proteins which interact with Chk1 may be identified by various assays.

Substrates of Chk1 may be identified by incorporating test compounds in assays for kinase activity. Chk1 kinase is resuspended in kinase buffer and incubated either in the presence or absence of the test compound (e.g., myelin basic protein, casein, histone H1, or appropriate substrate peptide). The amount of phosphate transferred by the kinase to the test compound are measured by autoradiography or scintillation counting. Transfer of phosphate to the test compound is indicative that the test compound is a substrate of the kinase.

Yet another aspect of this invention provides a diagnostic assay for detecting and quantifying the presence of Chk1 in a biological sample. A biological sample suspected of comprising Chk1 is utilized in a kinase assay. As described herein, the presence of Chk1 is identified by the phosphorylation of a substrate protein, e.g. myelin b protein, or the detection of a self-phosphorylated product. The phosphorylated product of the kinase reaction can be detected by for example, autoradiography or scintillation counting.

Interacting proteins may be identified by the following assays.

A first assay contemplated by the invention is a two-hybrid screen. The two-hybrid system was developed in yeast [Chien et al., Proc. Natl. Acad. Sci. USA, 88: 9578-9582 (1991)] and is based on functional in vivo reconstitution of a transcription factor which activates a reporter gene. Specifically, a polynucleotide encoding a protein that interacts with Chk1 is isolated by: transforming or transfecting appropriate host cells with a DNA construct comprising a reporter gene under the control of a promoter regulated by a transcription factor having a DNA binding domain and an activating domain; expressing in the host cells a first hybrid DNA sequence encoding a first fusion of part or all of Chk1 and either the DNA binding domain or the activating domain of the transcript ion factor; expressing in the host cells a library of second hybrid DNA sequences encoding second fusions of part or all of putative Chk1 binding proteins and the DNA binding domain or activating domain of the transcription factor which is not incorporated in the first fusion; detecting binding of an Chk1 interacting protein to Chk1 in a particular host cell by detecting the production of reporter gene product in the host cell; and isolating second hybrid DNA sequences encoding the interacting protein from the particular host cell. Presently preferred for use in the assay are a lexA promoter to drive expression of the reporter gene, the lacZ reporter gene, a transcription factor comprising the lexA DNA binding domain and the GAL4 transactivation domain, and yeast host cells.

Other assays for identifying proteins that interact with Chk1 may involve immobilizing Chk1 or a test protein, detectably labeling the nonimmobilized binding partner, incubating the binding partners together and determining the amount of label bound. Bound label indicates that the test protein interacts with Chk1.

Another type of assay for identifying Chk1 interacting proteins involves immobilizing Chk1 or a fragment thereof on a solid support coated (or impregnated with) a fluorescent agent, labeling a test protein with a compound capable of exciting the fluorescent agent, contacting the immobilized Chk1 with the labeled test protein, detecting light emission by the fluorescent agent, and identifying interacting proteins as test proteins which result in the emission of light by the fluorescent agent. Alternatively, the putative interacting protein may be immobilized and Chk1 may be labeled in the assay.

Also comprehended by the present invention are antibody products (e.g., monoclonal and polyclonal antibodies, single chain antibodies, chimeric antibodies, CDR-grafted antibodies and antigen-binding fragments thereof) and other binding proteins (such as those identified in the assays above) which are specific for the Chk1 kinases of the invention. Binding proteins can be developed using isolated natural or recombinant enzymes. The binding proteins are useful, in turn, for purifying recombinant and naturally occurring enzymes and identifying cells producing such enzymes. Assays for the detection and quantification of proteins in cells and in fluids may involve a single antibody substance or multiple antibody substances in a "sandwich" assay format to determine cytological analysis of Chk1 protein levels. The binding proteins are also manifestly useful in modulating (i.e.,blocking, inhibiting, or stimulating) enzyme/substrate or enzyme/regulator interactions. Anti-idiotypic antibodies specific for mammalian checkpoint kinase binding proteins are also contemplated.

It is further contemplated that antibodies against Chk1 can be used in diagnosis of Atm function. Because Chk1 protein levels are low or absent in AT patients Chk1 levels can be used as a marker in the development of compounds that inhibit Atm function. Because expression of Chk1 in AT cells is low or non-existent, inhibition of Atm function should decrease Chk1 expression. This decrease can be monitored by examining the expression levels of Chk1.

The invention contemplates that mutations in the Chk1 gene that result in loss of normal function of the Chk1 gene product underlie human disease states in which failure of a cell cycle checkpoint is involved. Gene therapy to restore Chk1 activity would thus be indicated in treating those disease states (for example, testicular cancer). Delivery of a functional Chk1 gene to appropriate cells is effected in vivo or ex vivo by use of viral vectors (e.g., adenovirus, adeno-associated virus, or a retrovirus) or ex vivo by use of physical DNA transfer methods (e.g., liposomes or chemical treatments). For reviews of gene therapy technology see Friedmann, Science, 244: 1275-1281 (1989); Verma, Scientific American: 68-84 (1990); and Miller, Nature, 357: 455-460 (1992). Alternatively, it is contemplated that in other human disease states preventing the expression of or inhibiting the activity of Chk1 will be useful in treating the disease states. It is contemplated that antisense therapy or gene therapy could be applied to negatively

regulate the expression of Chk1. Antisense nucleic acids (preferably 10 to 20 base pair oligonucleotides) capable of specifically binding to Chk1 expression control sequences or Chk1 RNA are introduced into cells (e.g., by a viral vector or colloidal dispersion system such as a liposome). The antisense nucleic acid binds to the Chk1 target sequence in the cell and prevents transcription or translation of the target sequence. Phosphothioate and methylphosphate antisense oligonucleotides are specifically contemplated for therapeutic use by the invention. The antisense oligonucleotides may be further modified by poly-L-lysine, transferrin polylysine, or cholesterol moieties at their 5 end.

Checkpoint signal transduction results in transcriptional regulation. One example of transcriptional regulation is MyoD muscle regulation. Chk1 expression suppresses the ability of MyoD to induce muscle gene transcription and suppresses the ability of MyoD to induce myogenesis (see Example 7). It is contemplated that another aspect of this invention is to regulate Chk1 levels in order to effect the differentiation and proliferation of stem cells such as those involved in muscle proliferation.

Agents that modulate Chk1 protein kinase activity may be identified by incubating a test compound with Chk1 immunopurified from cells naturally expressing the mammalian checkpoint protein kinase, with Chk1 obtained from recombinant procaryotic or eukaryotic host cells expressing the enzyme, or with purified Chk1, and then determining the effect of the test compound on Chk1 protein kinase activity. The activity of the checkpoint protein kinase can be measured by determining the amount of 32P-phosphate transferred by the protein kinase from gamma-32P-ATP to either itself (autophosphorylation) or to an exogenous substrate such as a lipid or protein. The amount of phosphate incorporated into the substrate is measured by scintillation counting or autoradiography. An increase in the amount of phosphate transferred to the substrate in the presence of the test compound compared to the amount of phosphate transferred to the substrate in the absence of the test compound indicates that the test compound is an activator of the Chk1 protein kinase. Conversely, a decrease in the amount of phosphate transferred to the substrate in presence of the test compound compared to the moles of phosphate transferred to the substrate in the absence of the test compound indicates that the modulator is an inhibitor of the Chk1 protein kinase.

In a presently preferred assay, a Chk1-specific antibody linked to agarose beads is incubated with a cell lysate prepared from host cells expressing the protein kinase. The beads are washed to remove proteins binding nonspecifically to the beads and the beads are then resuspended in kinase buffer. The reaction is initiated by the addition of gamma-32P-ATP and an appropriate exogenous substrate such as lipid or peptide. The activity of the protein kinase is measured by determining the moles of 32P-phosphate transferred either to the protein kinase itself or the added substrate.

In a preferred embodiment the host cells lack endogenous Chk1 and/or ATM protein kinase activity. The selectivity of a compound that modulates the protein kinase activity of Chk1 can be evaluated by comparing its activity on Chk1 to its activity on other known mammalian checkpoint protein kinases. The combination of the recombinant Chk1 products of the invention with other recombinant mammalian checkpoint kinase products in a series of independent assays provides a system for developing selective modulators of Chk1.

Furthermore, combinatorial libraries, peptide and peptide mimetics, defined chemical entities, oligonucleotides, and natural product libraries may be screened for activity as modulators in assays such as those described below.

For example, an assay for identifying modulators of Chk1 kinase activity involves incubating a Chk1 protein kinase preparation in kinase buffer with gamma-32P-ATP and an exogenous kinase substrate, both in the presence and absence of a test compound, and measuring the amount of phosphate transferred to the substrate. An increase in the amount of phosphate transferred to the substrate in presence of the test compound compared to the amount of phosphate transferred to the substrate in the absence of the test compound indicates that the test compound is an activator of the Chk1 kinase. Conversely, a decrease in the amount of phosphate transferred to the substrate in presence of the test compound compared to the amount of phosphate transferred to the substrate in the absence of the test compound indicates that the modulator is an inhibitor of said Chk1 protein kinase.

Moreover, assays for identifying compounds that modulate interaction of Chk1 with other proteins may involve: transforming or transfecting appropriate host cells with a DNA construct comprising a reporter gene under the control of a promoter

regulated by a transcription factor having a DNA-binding domain and an activating domain; expressing in the host cells a first hybrid DNA sequence encoding a first fusion of part or all of Chk1 and the DNA binding domain or the activating domain of the transcription factor; expressing in the host cells a second hybrid DNA sequence encoding part or all of a protein that interacts with Chk1 and the DNA binding domain or activating domain of the transcription factor which is not incorporated in the first fusion; evaluating the effect of a test compound on the interaction between Chk1 and the interacting protein by detecting binding of the interacting protein to Chk1 in a particular host cell by measuring the production of reporter gene product in the host cell in the presence or absence of the test compound; and identifying modulating compounds as those test compounds altering production of the reported gene product in comparison to production of the reporter gene product in the absence of the modulating compound. Presently preferred for use in the assay are a lexA promoter to drive expression of the reporter gene, the lacZ reporter gene, a transcription factor comprising the lexA DNA binding domain and the GAL4 transactivation domain, and yeast host cells.

Another type of assay for identifying compounds that modulate the interaction between Chk1 and an interacting protein involves immobilizing Chk1 or a natural Chk1 interacting protein, detectably labeling the nonimmobilized binding partner, incubating the binding partners together and determining the effect of a test compound on the amount of label bound wherein a reduction in the label bound in the present of the test compound compared to the amount of label bound in the absence of the test compound indicates that the test agent is an inhibitor of Chk1 interaction with protein. Conversely, an increase in the binding in the presence of the test compound compared to the amount label bound in the absence of the compound indicates that the putative modulator is an activator of Chk1 interaction with the protein.

Yet another method contemplated by the invention for identifying compounds that modulate the binding between Chk1 and an interacting protein involves immobilizing Chk1 or a fragment thereof on a solid support coated (or impregnated with) a fluorescent agent, labeling the interacting protein with a compound capable of exciting the fluorescent agent, contacting the immobilized Chk1 with the labeled interacting protein in the presence and absence of a test compound, detecting light emission by the

fluorescent agent, and identifying modulating compounds as those test compounds that affect the emission of light by the fluorescent agent in comparison to the emission of light by the fluorescent agent in the absence of the test compound. Alternatively, the Chk1 interacting protein may be immobilized and Chk1 may be labeled in the assay.

Modulators of Chk1 may affect its protein kinase activity, its localization in the cell, and/or its interaction with members of the cell cycle checkpoint pathway. Chk1 modulators may be formulated in compositions comprising pharmaceutically acceptable carriers. Such compositions may additionally include chemotherapeutic agents. Dosage amounts indicated would be sufficient to result in modulation of Chk1 activity in vivo. Selective modulators may include, for example, polypeptides or peptides which specifically bind to Chk1 or Chk1 nucleic acid, oligonucleotides which specifically bind to Chk1 or Chk1 nucleic acid, and/or other non-peptide compounds (e.g., isolated or synthetic organic molecules) which specifically react with Chk1 or Chk1 nucleic acid. Mutant forms of Chk1 which affect the enzymatic activity or cellular localization of wild-type Chk1 are also contemplated by the invention.

DETAILED DESCRIPTION

The present invention is illustrated by the following examples. Example 1 details the isolation of polynucleotides encoding mammalian Chk1 kinases and chromosomal mapping of the human Chk1 DNA. Example 2 describes the recombinant expression of DNAs encoding mammalian Chk1. Example 3 describes the preparation of antibodies to Chk1. Northern blots showing tissue and cell distribution of Chk1 are described in Example 4. Example 5 reports the results of immunohistological and western blot studies of Chk1 expression. Example 6 describes biochemical and biological activities of murine Chk1.

Example 1

A. Isolation of Human Chk1 cDNA

A Chk1Hu cDNA was identified by screening EST sequences for similarity to S. pombe Chk1. An EST (H67490) with homology to the COOH-terminus of Chk1 was identified and cloned and used to build a contig showing limited homology to the

COOH-terminal 120 amino acids. This contig was extended by RACE PCR to give a clone of 1735bp. The RACE PCR fragment was used to probe cDNA libraries to generate the final sequence. Library screening of 1 x 10^6 independent cDNA clones from a human testis unizap library (Clontech) with RACE derived sequence information at high stringency in Express hybridization solution (Clontech) yielded eleven overlapping sequences that were used to assemble the full length Chk1Hu.

The full length human Chk1 cDNA was subcloned into pGEMT. The plasmid containing the full length human cDNA is identified as pGEMT-ChklHU pGEMT-Chk1HU was deposited with American Type Culture Collection, 12301 Parklawn Drive, Rockville, MX 20852 on date 27th August 1997 under Accession No. ATCC 98520. The full length cDNA and deduced amino acid sequences of human Chk1 (Chk1Hu) are provided in SEQ ID NOs.: 2 and 4 respectively. The full length DNA and deduced amino acid sequences of human Chk1 (Chk1Hu) are provided in SEQ ID NOs.: 1 and 2, respectively.

B. Isolation of a Murine Chk1 cDNA

The Chk1Mo was identified by library screening of a mouse testis cDNA library using a degenerate human Chk1 specific probe. The partial cDNA and deduced amino acid sequences of mouse Chk1 (Chk1Mu) are provided in SEQ ID NO.: 3 and 4, respectively.

C. Structural Analysis of Chk1Hu and Chk1Mu

The Chk1Hu and Chk1Mu cDNAs encode a protein of approximately 70 kD. The kinase domain of Chk1Hu is 161 to 264 of SEQ ID NO.: 2. The kinase domain of Chk1Mu comprises amino acids 1 to 61 of SEQ ID NO.: 4. The protein kinase domains of human and mouse Chk1 as disclosed herein and are approximately 90% identical at the amino acid level. Relative to C. elegans, S. pombe, and S. cerevisiae Chk1-like proteins, the human form is approximately 56%, 47%, and 37% identical to the protein kinase domains, respectively. Figure 1 compares the amino acid sequences of Chk1 homologs from human, mouse, C. elegans, S. pombe and S. cerevisiae. In Figure 1, amino acid residues identical among all species are boxed. Roman numerals indicate subdomains

conserved among the homologs. Subdomains V through IX comprise the substrate recognition site and contain the greatest frequency of conserved residues. This is determined by homology with other known kinases (Hanks et al., Science 241:42-52, 1988).

5

D. Isolation of a Murine Chk1 Genomic Clone

A mouse genomic clone encoding Chk1 was obtained using PCR screening of a P1 mouse 129 library. The PCR primers used to screen the mouse 129 p1 library were mmChk2 (ACG TGG ACA AAC TGG TTC AGG) (SEQ ID NO.: 5) and mmChk 21: CTG ATA GCC CAA CTT CTC GAA SEQ ID NO.: 6). These primers were used to generate an amplicon of 208 bp corresponding to nucleotide X to X of SEQ ID NO.: 4. The amplicon was used to identify a clone of approximately 81-100 kb. An EcoR1 restriction digest of the genomic clone was performed and the restriction products were subcloned into a vector with zeocin as the selectable marker. The vector was obtained from Invitrogen p. zero 1.1 (2.8 kb).

The Eco RI restriction digests were resolved on a 0.8% agarose gel and transferred to nitrocellulose according to standard procedures. The nitrocellulose blot was probed with a 208bp amplicon to confirm that a genomic clone was identified.

E. Chromosomal Mapping of the Human Chk1 Gene

The Stanford G3 Radiation Hybrid panel was used to map the location of Chk1. PCR reactions using two oligonucleotides in the 3′ untranslated region of the human cDNA library derived Chk1 DNA fragment yielded a unique PCR amplicon with primer 1(Chk1 30mer3′UT) GGCTCTGGGGAATCCTGGTGAATATAGTGCTGC (SEQ ID NO.: 7 and primer 2 (Chk1 30mer 3′UT) TCCCCTGAAACTTGGTTTCCACCAGATGAG (SEQ ID NO. 8. For sublocalization, chromosome 11 radiation hybrid DNA samples obtained from Research Genetics (Huntsville, Alabama) were analyzed and the results were decoded by the RH server at http://shgc.stanford.edu/. Chk1Hu localized to marker D1154610 which has been mapped to the telomeric region of 11q at 23.3. This region has been identified as the site of tumor

suppressor genes implicated in ovarian, breast, lung, colon and cervix cancer and melanoma. [Gabra et al., Cancer Research, 56:950-954 (1996)].

Example 2

Chk1 was expressed in recombinant host cells.

A. Expression and Kinase Activity of Chk1 Glutathione-S-Transferase Fusion Protein

DNA encoding Chk1 glutathione-S-transferase fusion protein (GST-Chk1Hu) was also prepared. A DNA encoding GST-Chk1Hu was cloned into pGEX KGH using NdeI SalI restriction sites introduced into Chk1Hu. The internal Nde I sites were eliminated by silent mutagenesis. E. coli F Dh5a transformed with GST-Chk1 were used to prepare recombinant proteins. To 200 ml of culture, 5mM IPTG was added and cells were grown for four hours at 37 C. The culture was harvested and washed in STE buffer (10mM Tris pH 8, 150 mM NaCl, and 1mM EDTA). The cells were resuspended in 6 mol STE with 1mM PMSF and 100mg lysozyme. The cultures were incubated on ice for fifteen minutes, and 5 mM DTT and 1.5% Sarkosyl was added and the cells were sonicated. The debris was pelleted, and the supernatant was made to 2% Triton X-100. Glutathione agarose beads were added and the beads were pelleted in a benchtop centrifuge and bound proteins were eluted with elution buffer (50mM Tris pH 8.0, 15 0 mM NaCl, 1mM PMSF, and 10 mM glutathione.

Kinase assays were performed by incubating GST-Chk1Hu in kinase buffer (25M HEPES, pH 7.7; 50 mM KC1; 10 mM MgC12; 0.1% NP-40; 2% glycerol; 1mM DTT, 50uM ATP) with or without 1mg os substrate protein, and incubated in kinase buffer containing 10mCi [g-32P] ATP (3000 Ci/mmol) for twenty minutes at 37 C. The reactions were stopped with 20 ul 2 X SDS sample buffer prior to separation on 6% PAGE. Kinase reactions were then transferred to Immobilon, exposed to film, then subsequently probed to detect precipitated protein.

The glutathione affinity purified fusion was able to autophosphorylate and phosphorylate substrate proteins such a s myeline basic protein showing that Chk1 is active as a protein kinase, independent of regulatory subunits.

Example 3

Antibodies specific for mammalian Chk1 proteins were generated as follows.

A. Generation of Polyclonal Antibodies

Polyclonal antibodies were generated against a mouse Chk1 polypeptide fragment. CLKETFEKLGYQWKK (amino acids 191 to 203 of SEQ ID NO.: 3) was coupled to Keyhole Lympet Hemocyanin (KLH) via the N-terminally added cysteine and a rabbit was injected with 150 mg per injection. To affinity purify the rabbit sera, 3 mls of thiol coupling gel (TCGel Quality Controlled Biochemicals) (St. Louse, Missouri) that was equilibrated with degassed TEB (50 mM Tris, 5 mM EDTA-Na, pH 8.5) was mixed with 1.25 mg of HPLC purified peptide. The coupled resin was loaded into an econo column (Bio Rad) (Hercules, California) and was washed with 10 column volumes of TEB. The resin was treated blocking buffer (50 mM cysteine in TEB buffer per ml of gel) and was sequentially washed with 20 column volumes of salt buffer. The column was washed with 20 column volumes of salt buffer (500 mM NaC1 in 50 mM NaH2PO4, pH 6.5) and 10 column volumes of phosphate buffer (50 mM NaH2PO4, pH 6.5). Twenty mls of rabbit serum was loaded onto the column and was washed with 10 column volumes of salt buffer and the antibody was eluted with glycine buffer (100mM Glycine-HCL, pH 2.5). One ml fractions were collected in 50 ul of 1.0M Tris, (pH 9.5). Fractions containing antibody were pooled and dialyzed in storage buffer (10mM NaH2PO4, 20mM MgC1, pH 7.0) and stored at -20 as a Chk1#2-3. The polyclonal antisera, a Chk1#2-3 was able to immunoprecipitate mouse Chk1 protein from mouse testes extract as described in Example 6. In addition, aChk1#2-3 recognized recombinant human Chk1 and the Chk1Hu glutathione-S-transferase fusion protein expressed in E. coli.

B. Generation of Monoclonal Antibodies

Monoclonal antibodies are prepared by immunizing Balb/c mice subcutaneously with Chk1, Gst-Chk1 or a Chk1 fragment in complete Freund's adjuvant (CFA). Subsequent immunizations in CFA or incomplete Freund's adjuvant is performed to increase immune response.

The spleen of the immunized animal is removed aseptically and a single-cell suspension is formed by grinding the spleen between the frosted ends of two glass microscope slides submerged in serum free RPMI 1640, supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, 100 units/ml penicillin, and 100 mg/ml streptomycin (RPMI) (Gibco, Canada). The cell suspension is filtered through sterile 70-mesh Nitex cell strainer (Becton Dickinson, Parsippany, New Jersey), and washed twice by centrifuging at 200 g for 5 minutes and resuspending the pellet in 20 ml serum free RPMI. Thymocytes taken from naive Balb/c mice are prepared in the same manner.

Two x 108 spleen cells are combined with 4 x 107 NS-1 cells (kept in log phase in RPMI with 11% fetal bovine serum (FBS) for three days prior to fusion), centrifuged and the supernatant is aspirated. The cell pellet is dislodged and 2 ml of 37 C PEG 1500 (50% in 75 mM HEPES, pH 8.0) (Boehringer Mannheim) is added while stirring over the course of one minute, followed by the addition of 14 ml of serum free RPMI over seven minutes. Additional RPMI can be added and the cells are centrifuged at 200 g for 10 minutes. After discarding the supernatant, the pellet is resuspended in 200 ml RPMI containing 15% FBS, 100 mM sodium hypoxanthine, 0.4 mM aminopterin, 16 mM thymidine (HAT) (Gibco), 25 units/ml IL-6 (Boehringer Mannheim) and 1.5 x 106 thymocytes/ml. The suspension is dispensed into ten 96-well flat bottom tissue culture plates (Corning, United Kingdom) at 200 ml/well. Cells are fed on days 2, 4, and 6 days post-fusion by aspirating 100 ml from each well with an 18 G needle (Becton Dickinson), and adding 100 ml/well plating medium containing 10 U/ml IL-6 and lacking thymocytes.

When cell growth reaches 60-80% confluence (day 8-10), culture supernatants are taken from each well and screened for reactivity to Chk1 by ELISA. ELISAs are performed as follows. Immulon 4 plates (Dynatech, Cambridge, Massachusetts) are coated at 4 C with 50 ml/well with 100ng/well of Chk1 in 50 mM carbonate buffer, pH 9.6. Plates are washed with PBS with 0.05%, Tween 20 (PBST) and blocked 30 minutes at 37 C with 0.5% Fish Skin Gelatin. Plates are washed as described above and 50 ml culture supernatant is added. After incubation at 37 C for 30 minutes, 50 ml of horseradish peroxidase conjugated goat anti-mouse IgG(fc) (Jackson ImmunoResearch, West Grove, Pennsylvania) [diluted 1:10,000 in PBST] is added. Plates are incubated at 37 C for 30 minutes, washed with PBST and 100 ml of substrate,

consisting of 1 mg/ml TMB (Sigma) and 0.15ml/ml 30% H2O2 in 100 mM Citrate, pH 4.5, is added. The color reaction is stopped with the addition of 50 ml of 15% H2SO4. A450 is read on a plate reader (Dynatech).

Example 4

Northern analysis was performed to determine tissue distribution of Chk1 in mouse and human tissue. Oligonucleotides mmchk1: GTTGAGACTCCATCATCAAGG (SEQ ID NO.: 9) and mmChk1': TCTGGCTGGGAACTAGAGAAC (SEQ ID NO.: 10) were used to generate an amplicon of 220bp, identified as mmChk1+1'. mmChk1+1' and mmChk2+2' (Example 1) were used as probes for northern analysis. The conditions for PCR were as follows: One cycle of eight minutes at 94°C then forty cycles of 94°C for twenty seconds, 58°C for twenty seconds, 72°C for twenty seconds. The products were analyzed on a 4% agarose gel.

The amplicons were labeled with 32P-ATP for Northern analysis. A nylon membrane containing 2mg of size fractionated poly (A)+ RNA from human and mouse tissue sources including human heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, colon, and peripheral blood leukocytes, and mouse heart, brain, spleen, lung, liver, skeletal muscle, kidney, and testis (Clonetech Laboratories, Palo Alto, California) was probed with the labeled amplicons as recommended by the manufacturer except that the final wash was performed at 55°C to minimize the possibility of cross-hybridization to related sequences.

In mouse tissue, Chk1 expression was observed in lung, spleen, and testes in the mouse. In human tissue, expression was seen in thymus, lung, prostate, and testes. However, testes RNA samples from both mouse and human show approximately two to four fold higher levels of RNA expression than other tissues.

To determine Chk1 expression in developing mouse embryos, mRNA obtained from total embryos at day 7, 11, 15, and 17 were probed with labeled mChk1+1' and mmChk2+2'. The Northern blots showed that Chk1 expression peaked at day 11 of embryogenesis.

Example 5

Because of the roles Atr and Atm play in meiosis and their association with meiotic chromosomes, the expression of Chk1Mo protein was examined by immunohistological characterization of cross-sections of mouse testes. In testes, pre-meiotic cells surround the surface of the seminiferous tubule and progression towards the interior lumen of the tubule corresponds to progressively later stages of meiosis and sperm maturation [Moens et al., J. Histochem. Cytochem, 25:480 (1977)].

Testes from normal and mutant mice (discussed infra) as described in Heiner et al., Cancer Res., 57:1664-1667 (1997) were obtained and preserved in Tissue Tek OCT compound, a tissue freezing media, containing 10.24% w/w polyvinyl alcohol, 4.26% w/w polyethylene glycol, and 85.50% w/w nonreacting ingredients. Both wildtype and mutant mice were tested and analyzed by immunohistochemistry using aChk1#2-3, a polyclonal antibody. Six micron cryosections were placed in a 50 C oven to dry and fixed in 4 C acetone for two minutes. The slides were incubated with aChk1#2-3 antisera at a 1:100 dilution for thirty minutes. The secondary, goat anti-rabbit biotinylated antibody was applied to each section at a dilution of 1:200 and incubated for fifteen minutes at 37 C. The tertiary antibody, goat anti biotin was applied to each section at a dilution of 1:200 and incubated for fifteen minutes at 37 C. After each incubation, the slides were rinsed with 1 X TBS. DAB horseradish-peroxidase substrate was used to detect positive signal in the samples. The reaction was stopped in water and counterstained with Gill's Hematoxylin.

The testes of Chk1Mo, atm+/+ p53+/+ (A), atm+/+ p53+/- (B) atm-/- p53+/+ (C), atm-/- p53+/- (D), and, atm-/- p53-/- (E) were immunohistologically characterized using anti-Chk1 antiserum. Testes cryosections were stained with affinity-purified anti-Chk1 antiserum (aChk1 #2-3) and with an anti-ATR monoclonal (224C). The specificity of staining was confirmed by examining pre-immune serum and by specific and non-specific peptide block experiments. Affinity purified Chk1 polyclonal antisera (Chk1 #2-3 of Example 3) was used to determine the localization.

In contrast to staining patterns reported for Atr, Chk1Mo shows temporal increases and decreases in nuclear staining in normal mice. Chk1Mo is most highly expressed at pachynema in primary spermatocytes, indicating that Chk1Mo may act

downstream of Atr function during meiotic prophase, after Atr, which acts earlier during zygonema. Since Atm and p53 have checkpoint properties and may act at an earlier phase in signaling relative to Chk1Hu/Mo, the localization of Chk1Mo by histological analyzes was examined in atm-/-p53+/+, atm-/-p53-/- and atm-/-p53+/- mice [Done hower et al., Nature, 356:215 (1992); Kuerbitz et al., Proc. Natl. Acad. Sci. USA, 89:7491 (1992); Westphal et al., Nat. Gen., 16:397-401 (1997)]. Chk1Mo accumulation and localization was independent of p53 status but dependent on Atm, suggesting that Chk1Mo also acts downstream of Atm in meiotic prophase.

To further analyze the role of Chk1 in mammalian meiosis, the temporal and spatial distribution of Chk1 in surface spread preparations of spermatocytes was determined.

For the meiotic preparations, surface spreads of spermatocytes from fifteen to twenty-one day old mice (C57-bl/6) were prepared and antibody incubation and detection procedures were preformed as described in Ashley et al., Chromosoma, 104:19 (1995). Antibody incubation and detection procedures were a modification of the protocol of Moens et al. as described previously in Ashley et al. Since the antibodies against Chk1 and Sep3 (control) were both raised in rabbit, spermatocytes were labeled and imaged sequentially. Goat-anti rabbit IgG-rhodamine-conjugated and goat-anti rabbit IgG-FITC-conjugated (Pierce) secondary antibodies were used for detection. All preparations were counterstained with 4′, 6′ diamino-2-phenylindole (DPAI, Sigma) and mounted in a DABCO (Sigma) antifade solution. The preparations were examined on a Zeiss Axioskop (63-X and 100-X, 1.2 Plan Neoflour oil-immersion objective). Each fluorochrome (FITC, rhodamine and DAPI) image was captured separately as an 8-bit source image using a computer assisted cooled CCD camera (Photometrics CH220) and the separate images 24-bit pseudocolored and merged with custom software developed by Tim Rand [Ried et al., Proc. Natl. Acad. Sci. USA, 89:1388 (1992)].

Zygotene spermatocytes were stained with an antiserum against Scp3, a component of the axial element, which forms between the sister-chromatids. The same zygotene spermatocytes were labeled with a-Chk1#23. Chk1 is present along the synaptonemal complexes (SC) of synapsed homologous chromosomes. Chromosomes that are in the process of synapsing have Chk1 staining, but no staining is observed on the

unsynapsed axial elements. As meiosis proceeds into pachynema, Chk1Mo remains associated with autosomal synaptonemal complexes in a focal staining pattern similar to ATM.

A pachytene spermatocyte was labeled with both anti-Chk1 and a mouse monoclonal antibody against Atr (224C). Although Chk1 initially appears in a focal pattern, in pachynema Chk1 seems to accumulate along the SCs. In addition, Chk1 foci appear along the unsynapsed axial elements of the X and Y chromosomes in mid pachynema where it colocalizes with Atr. Chk1 remains on the SCs throughout pachynema and disappears when the homologous chromosomes disassociate in diplonema.

Progression of meiotic prophase I appears to be normal in p53-/- mice. In an early pachytene spermatocyte labeled for Chk1 and Scp3, Chk1 is present along the SCs and the synapsed region of the sex chromosomes. However, Chk1 does not appear along the unsynapsed axial element of the X chromosome in early pachytene. In atm-/- mice, progression of meiosis is disrupted as the SCs begin to fragment following synapsis. Although homologous chromosomes synapse in atm-/- spermatocytes, no Chk1 is detected along the synapsed bivalents or fragmented SCs.

Thus, in normal mice, Chk1 appears in a focal pattern along the synaptonemal complexes of synapsing homologous chromosomes in zygonema, in a pattern similar to that of Atm. Chk1 accumulates along the SCs as meiosis progresses into pachynema, and by mid-pachynema Chk1 coats the entire SCs. In early pachynema, Chk1 is present along the synapsed region of the XY bivalent. However, by mid-pachynema, Chk1 foci also appear along the unsynapsed axes of both the X and Y chromosomes, where it colocalizes with Atr. Atr is found in foci along the unsynapsed axes of the sex chromosomes early, and later coats the entire X and Y axes throughout pachynema.

Meiosis appears to be unaffected in p53 deficient mice, as is demonstrated by histological analysis and immunostaining of surface spread spermatocytes with Scp3 and Chk1. In contrast, atm-/- mice are sterile as the result of progressive fragmentation of meiotic chromosomes following synapsis, leading to apoptosis. Immunolocalization of Chk1 in atm-/- spermatocytes indicates a lack of Chk1 on the SCs, suggesting a role of Chk1 downstream of Atm in mammalian meiosis. To determine if the lack of Chk1

staining in atm-/- nuclei and the lack of Chk1Mo protein on meiotic prophase chromatin was reflected by the level of Chk1Mo, Western analysis of testes extracts was performed according to Keegan et al., Genes Dev., 10:2423 (1996). Chk1 protein was present in an Atm-dependent fashion, suggesting that synthesis or stability of Chk1Mo depends on the Atm protein. MTE of mice lacking Atm did not stain for Chk1 indicating that atm-1- mice did not express Chk1. In contrast, MTE of wild type mouse or from mice in which p53 expression was disrupted showed Chk1 staining.

Example 6

The kinase activity of mouse Chk1 and its ability to associate with Atr were also demonstrated.

A. Kinase Activity of Murine Testes Chk1

Antibody aChk1#2-3 also used to immunoprecipitate Chk1 from mouse testes extract (MTE). Approximately 30 decapsulated testes were ground in a mortar with liquid nitrogen and the grounds were transferred to a 15 ml dounce homogenizer. Fifteen mls of lysis buffer (50mM NaPO4, pH 7.2, 0.5% TritonX-100, 2mM EDTA, 2mM EGTA, 25mM NaF, 25mM 2-glycerophosphate, 1mM phenylmethylsulfonyl fluoride (PMSF), 1mg/ml. leupeptin, 1 mg/ml pepstatin A, and 2mM DTT) was added and the extract was dounced 30 times with a loose pestle and 20 times with a tight pestle. After a low speed spin, the supernatant assayed using BCA for protein concentration determination.

For Chk1 immunoprecipitations, 400mg of MTE extract was incubated with either 10mg of affinity purified Chk1#2-3 or with approximately 10mg of preimmune serum for thirty minutes on ice. Protein A-agarose slurry (Pierce) was added and the mixture was incubated for thirty minutes at 4 C. The immune-complex bound slurry was washed three times in TSAT and one time with kinase buffer (25uM HEPES, pH 7.7; 50 mM KC1; 10 mM MgC12; 0.1% NP-40; 2% glycerol; 1mM DTT, 50uM ATP), and incubated in kinase buffer containing 10mCi [g-32P] ATP (3000 Ci/mmol) for twenty minutes at 37 C. The reactions were stopped with 20 ul 2 X SDS sample buffer prior to

separation on 6% PAGE. Kinase reactions were then transferred to Immobilon, exposed to film, then subsequently probed to detect precipitated protein.

To determine if the immunoprecipitated Chk1 from MTE could self-phosphorylate, 2X kinase buffer and 10 mCi g32-P-data (3000 Ci/mM) were added to the immunoprecipitate. The phosphorylation reactions were incubated at 30 C for fifteen minutes. The reactions were electrophoresed on a 6% PAGE gel, transferred to immobilon P, and exposed to X-ray film. The blots showed that immunoprecipitated Chk1 was able to self phosphorylate as was the Chk1-GST fusion protein (Example 2). Mouse IgG and Chk1 preimmune sera did not immunoprecipitate Chk1Mo.

C. Association of Chk1 and Atr

To determine if Chk1 and Atr can associate in meiotic cells, 460 mg of MTE was immunoprecipitated with anti-Atr monoclonal antibody (aAtr-224C) under conditions as described above. The Atr immunoprecipitate was electrophoresed on a 6% or 8% PAGE, electroblotted onto immoblin P membrane and was probed with anti-Chk1 antibody (aChk1 #2-3). The blots showed that Chk1 co-precipitates with Atr indicating that Atr and Chk1 associate in meiotic cells. In addition, the Chk1 that immunoprecipitates with Atr was able to self phosphorylate.

Numerous modifications and variations in the practice of this invention are expected to occur to those of skill in the art. Only such limitations that appear in the appended claims should be placed on the invention.

CLAIMS

We claim:

1. A purified and isolated polynucleotide encoding the human Chk1 kinase amino acid sequence set out in SEQ ID NO.: 2.

2. A purified and isolated polynucleotide encoding the mouse Chk1 kinase amino acid sequence set out in SEQ ID NO.: 4.

3. The polynucleotide of claim 1 or 2 which is a DNA.

4. The DNA of claim 3 which is a cDNA.

5. The DNA of claim 3 which is a genomic DNA.

6. The DNA of claim 3 which is a wholly or partially chemically synthesized DNA.

7. A human Chk1 DNA comprising the DNA sequence set out in SEQ ID NO.: 1.

8. A mouse Chk1 DNA comprising the DNA sequence set out in SEQ ID NO.: 3.

9. An RNA transcript of the DNA of claim 3.

10. A DNA encoding a full length mammalian Chk1 kinase selected from the group consisting of:

a) a DNA which hybridizes under stringent conditions to the non-coding strand of the DNA of SEQ ID NO.: 2; and

b) a DNA which hybridizes under stringent conditions to the non-coding strand of the DNA of SEQ ID NO.: 4.

11. A vector comprising a DNA according to claims 1, 2, 3 or 10.

12. The vector of claim 11 wherein said DNA is operatively linked to an expression control DNA sequence.

13. A host cell stably transformed or transfected with a DNA according to claims 1, 2, 3 or 10 in a manner allowing the expression in said host cell of the Chk1 kinase.

14. A method for producing Chk1 kinase, said method comprising growing a host cell according to claim 11 in a suitable nutrient medium and isolating the Chk1 kinase.

15. A purified and isolated polypeptide comprising the human Chk1 kinase amino acid sequence consisting of SEQ ID NO.: 2.

16. A purified and isolated polypeptide comprising the mouse Chk1 kinase amino acid sequence consisting of SEQ ID NO.: 4.

17. A polypeptide or peptide capable of specifically binding to mammalian Chk1 kinase.

18. The polypeptide according to claim 17 which is an antibody.

19. The antibody according to claim 18 which is a monoclonal antibody.

20. A hybridoma cell line producing the monoclonal antibody according to claim 19.

21. A method of identifying a compound that is a modulator of mammalian Chk1 kinase comprising the steps of:

a) determining the kinase activity of Chk1 in the presence and absence of said compound;

b) comparing the kinase activities observed in step (a); and

c) identifying said compound as a modulator by the observed differences in the kinase activity of Chk1 in the presence and absence of said compound.

22. A method of identifying a compound that inhibits mammalian Chk1 comprising the steps of:

a) expressing mammalian Chk1 in a genetically altered cell, thereby decreasing the sensitivity of the cell to DNA damage, said sensitivity being associated with the genetic alteration;

b) exposing the genetically altered cell of step (a) to DNA damaging treatment in the presence and absence of a test modulator compound;

c) measuring the sensitivity of the cell to DNA damage; and

d) identifying a test compound that restores the sensitivity of the cell to DNA damage as an inhibitor of Chk1 activity.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Medical Research Council

(ii) TITLE OF INVENTION: MAMMALIAN CHK1 EFFECTOR CELL-CYCLE CHECKPOINT PROTEIN KINASE MATERIALS AND METHODS

(iii) NUMBER OF SEQUENCES: 10

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Medical research Council
(B) STREET: 20, Park Crescent
(C) CITY: London
(D) STATE:
(E) COUNTRY: UK
(F) ZIP: W1N 4AL

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1933 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
"Human Chk1"

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 34..1461

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
GCCGGACAGT CCGCCGAGGT GCTCGGTGGA GTC ATG GCA GTG CCC TTT GTG GAA   54
                                     Met Ala Val Pro Phe Val Glu
                                       1               5

GAC TGG GAC TTG GTG CAA ACC CTG GGA GAA GGT GCC TAT GGA GAA GTT   102
Asp Trp Asp Leu Val Gln Thr Leu Gly Glu Gly Ala Tyr Gly Glu Val
         10                  15                  20
```

```
CAA CTT GCT GTG AAT AGA GTA ACT GAA GAA GCA GTC GCA GTG AAG ATT      150
Gln Leu Ala Val Asn Arg Val Thr Glu Glu Ala Val Ala Val Lys Ile
 25                  30                  35

GTA GAT ATG AAG CGT GCC GTA GAC TGT CCA GAA AAT ATT AAG AAA GAG      198
Val Asp Met Lys Arg Ala Val Asp Cys Pro Glu Asn Ile Lys Lys Glu
 40                  45                  50                  55

ATC TGT ATC AAT AAA ATG CTA AAT CAT GAA AAT GTA GTA AAA TTC TAT      246
Ile Cys Ile Asn Lys Met Leu Asn His Glu Asn Val Val Lys Phe Tyr
                 60                  65                  70

GGT CAC AGG AGA GAA GGC AAT ATC CAA TAT TTA TTT CTG GAG TAC TGT      294
Gly His Arg Arg Glu Gly Asn Ile Gln Tyr Leu Phe Leu Glu Tyr Cys
             75                  80                  85

AGT GGA GGA GAG CTT TTT GAC AGA ATA GAG CCA GAC ATA GGC ATG CCT      342
Ser Gly Gly Glu Leu Phe Asp Arg Ile Glu Pro Asp Ile Gly Met Pro
         90                  95                  100

GAA CCA GAT GCT CAG AGA TTC TTC CAT CAA CTC ATG GCA GGG GTG GTT      390
Glu Pro Asp Ala Gln Arg Phe Phe His Gln Leu Met Ala Gly Val Val
 105                 110                 115

TAT CTG CAT GGT ATT GGA ATA ACT CAC AGG GAT ATT AAA CCA GAA AAT      438
Tyr Leu His Gly Ile Gly Ile Thr His Arg Asp Ile Lys Pro Glu Asn
120                 125                 130                 135

CTT CTG TTG GAT GAA AGG GAT AAC CTC AAA ATC TCA GAC TTT GGC TTG      486
Leu Leu Leu Asp Glu Arg Asp Asn Leu Lys Ile Ser Asp Phe Gly Leu
                140                 145                 150

GCA ACA GTA TTT CGG TAT AAT AAT CGT GAG CGT TTG TTG AAC AAG ATG      534
Ala Thr Val Phe Arg Tyr Asn Asn Arg Glu Arg Leu Leu Asn Lys Met
            155                 160                 165

TGT GGT ACT TTA CCA TAT GTT GCT CCA GAA CTT CTG AAG AGA AGA GAA      582
Cys Gly Thr Leu Pro Tyr Val Ala Pro Glu Leu Leu Lys Arg Arg Glu
        170                 175                 180

TTT CAT GCA GAA CCA GTT GAT GTT TGG TCC TGT GGA ATA GTA CTT ACT      630
Phe His Ala Glu Pro Val Asp Val Trp Ser Cys Gly Ile Val Leu Thr
 185                 190                 195

GCA ATG CTC GCT GGA GAA TTG CCA TGG GAC CAA CCC AGT GAC AGC TGT      678
Ala Met Leu Ala Gly Glu Leu Pro Trp Asp Gln Pro Ser Asp Ser Cys
200                 205                 210                 215

CAG GAG TAT TCT GAC TGG AAA GAA AAA AAA ACA TAC CTC AAC CCT TGG      726
Gln Glu Tyr Ser Asp Trp Lys Glu Lys Lys Thr Tyr Leu Asn Pro Trp
                220                 225                 230

AAA AAA ATC GAT TCT GCT CCT CTA GCT CTG CTG CAT AAA ATC TTA GTT      774
Lys Lys Ile Asp Ser Ala Pro Leu Ala Leu Leu His Lys Ile Leu Val
            235                 240                 245

GAG AAT CCA TCA GCA AGA ATT ACC ATT CCA GAC ATC AAA AAA GAT AGA      822
```

```
Glu Asn Pro Ser Ala Arg Ile Thr Ile Pro Asp Ile Lys Lys Asp Arg
        250                 255                 260

TGG TAC AAC AAA CCC CTC AAG AAA GGG GCA AAA AGG CCC CGA GTC ACT      870
Trp Tyr Asn Lys Pro Leu Lys Lys Gly Ala Lys Arg Pro Arg Val Thr
    265                 270                 275

TCA GGT GGT GTG TCA GAG TCT CCC AGT GGA TTT TCT AAG CAC ATT CAA      918
Ser Gly Gly Val Ser Glu Ser Pro Ser Gly Phe Ser Lys His Ile Gln
280                 285                 290                 295

TCC AAT TTG GAC TTC TCT CCA GTA AAC AGT GCT TCT AGT GAA GAA AAT      966
Ser Asn Leu Asp Phe Ser Pro Val Asn Ser Ala Ser Ser Glu Glu Asn
                300                 305                 310

GTG AAG TAC TCC AGT TCT CAG CCA GAA CCC CGC ACA GGT CTT TCC TTA     1014
Val Lys Tyr Ser Ser Ser Gln Pro Glu Pro Arg Thr Gly Leu Ser Leu
            315                 320                 325

TGG GAT ACC AGC CCC TCA TAC ATT GAT AAA TTG GTA CAA GGG ATC AGC     1062
Trp Asp Thr Ser Pro Ser Tyr Ile Asp Lys Leu Val Gln Gly Ile Ser
        330                 335                 340

TTT TCC CAG CCC ACA TGT CCT GAT CAT ATG CTT TTG AAT AGT CAG TTA     1110
Phe Ser Gln Pro Thr Cys Pro Asp His Met Leu Leu Asn Ser Gln Leu
    345                 350                 355

CTT GGC ACC CCA GGA TCC TCA CAG AAC CCC TGG CAG CGG TTG GTC AAA     1158
Leu Gly Thr Pro Gly Ser Ser Gln Asn Pro Trp Gln Arg Leu Val Lys
360                 365                 370                 375

AGA ATG ACA CGA TTC TTT ACC AAA TTG GAT GCA GAC AAA TCT TAT CAA     1206
Arg Met Thr Arg Phe Phe Thr Lys Leu Asp Ala Asp Lys Ser Tyr Gln
                380                 385                 390

TGC CTG AAA GAG ACT TGT GAG AAG TTG GGC TAT CAA TGG AAG AAA AGT     1254
Cys Leu Lys Glu Thr Cys Glu Lys Leu Gly Tyr Gln Trp Lys Lys Ser
            395                 400                 405

TGT ATG AAT CAG GTT ACT ATA TCA ACA ACT GAT AGG AGA AAC AAT AAA     1302
Cys Met Asn Gln Val Thr Ile Ser Thr Thr Asp Arg Arg Asn Asn Lys
        410                 415                 420

CTC ATT TTC AAA GTG AAT TTG TTA GAA ATG GAT GAT AAA ATA TTG GTT     1350
Leu Ile Phe Lys Val Asn Leu Leu Glu Met Asp Asp Lys Ile Leu Val
    425                 430                 435

GAC TTC CGG CTT TCT AAG GGT GAT GGA TTG GAG TTC AAG AGA CAC TTC     1398
Asp Phe Arg Leu Ser Lys Gly Asp Gly Leu Glu Phe Lys Arg His Phe
440                 445                 450                 455

CTG AAG ATT AAA GGG AAG CTG ATT GAT ATT GTG AGC AGC CAG AAG GTT     1446
Leu Lys Ile Lys Gly Lys Leu Ile Asp Ile Val Ser Ser Gln Lys Val
                460                 465                 470

TGG CTT CCT GCC ACA TGATCGGACC ATCGGCTCTG GGGAATCCTG GTGAATATAG     1501
Trp Leu Pro Ala Thr
```

475

TGCTGCTATG TTGACATTAT TCTTCCTAGA GAAGATTATC CTGTCCTGCA AACTGCAAAT 1561

AGTAGTTCCT GAAGTGTTCA CTTCCCTGTT TATCCAAACA TCTTCCAATT TATTTTGTTT 1621

GTTCGGCATA CAAATAATAC CTATATCTTA ATTGTAAGCA AAACTTTGGG GAAAGGATGA 1681

ATAGAATTCA TTTGATTATT TCTTCATGTG TGTTTAGTAT CTGAATTTGA AACTCATCTG 1741

GTGGAAACCA AGTTTCAGGG GACATGAGTT TTCCAGCTTT TATACACACG TATCTCATTT 1801

TTATCAAAAC ATTTTGTTTA ATTCAAAAAG TACATATTCC ATGTTGATTT AATTCTAAGA 1861

TGAACCAATA AAGACATAAT TCTTGTGACT TTTGGACAGT AGATTTATCA GTCTGTGAAG 1921

CGAAGCCAGC TT 1933

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 476 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ala Val Pro Phe Val Glu Asp Trp Asp Leu Val Gln Thr Leu Gly
1 5 10 15

Glu Gly Ala Tyr Gly Glu Val Gln Leu Ala Val Asn Arg Val Thr Glu
20 25 30

Glu Ala Val Ala Val Lys Ile Val Asp Met Lys Arg Ala Val Asp Cys
35 40 45

Pro Glu Asn Ile Lys Lys Glu Ile Cys Ile Asn Lys Met Leu Asn His
50 55 60

Glu Asn Val Val Lys Phe Tyr Gly His Arg Arg Glu Gly Asn Ile Gln
65 70 75 80

Tyr Leu Phe Leu Glu Tyr Cys Ser Gly Gly Glu Leu Phe Asp Arg Ile
85 90 95

Glu Pro Asp Ile Gly Met Pro Glu Pro Asp Ala Gln Arg Phe Phe His
100 105 110

Gln Leu Met Ala Gly Val Val Tyr Leu His Gly Ile Gly Ile Thr His
115 120 125

Arg Asp Ile Lys Pro Glu Asn Leu Leu Leu Asp Glu Arg Asp Asn Leu
130 135 140

Lys Ile Ser Asp Phe Gly Leu Ala Thr Val Phe Arg Tyr Asn Asn Arg

145 150 155 160

Glu Arg Leu Leu Asn Lys Met Cys Gly Thr Leu Pro Tyr Val Ala Pro
165 170 175

Glu Leu Leu Lys Arg Arg Glu Phe His Ala Glu Pro Val Asp Val Trp
180 185 190

Ser Cys Gly Ile Val Leu Thr Ala Met Leu Ala Gly Glu Leu Pro Trp
195 200 205

Asp Gln Pro Ser Asp Ser Cys Gln Glu Tyr Ser Asp Trp Lys Glu Lys
210 215 220

Lys Thr Tyr Leu Asn Pro Trp Lys Lys Ile Asp Ser Ala Pro Leu Ala
225 230 235 240

Leu Leu His Lys Ile Leu Val Glu Asn Pro Ser Ala Arg Ile Thr Ile
245 250 255

Pro Asp Ile Lys Lys Asp Arg Trp Tyr Asn Lys Pro Leu Lys Lys Gly
260 265 270

Ala Lys Arg Pro Arg Val Thr Ser Gly Gly Val Ser Glu Ser Pro Ser
275 280 285

Gly Phe Ser Lys His Ile Gln Ser Asn Leu Asp Phe Ser Pro Val Asn
290 295 300

Ser Ala Ser Ser Glu Glu Asn Val Lys Tyr Ser Ser Ser Gln Pro Glu
305 310 315 320

Pro Arg Thr Gly Leu Ser Leu Trp Asp Thr Ser Pro Ser Tyr Ile Asp
325 330 335

Lys Leu Val Gln Gly Ile Ser Phe Ser Gln Pro Thr Cys Pro Asp His
340 345 350

Met Leu Leu Asn Ser Gln Leu Leu Gly Thr Pro Gly Ser Ser Gln Asn
355 360 365

Pro Trp Gln Arg Leu Val Lys Arg Met Thr Arg Phe Phe Thr Lys Leu
370 375 380

Asp Ala Asp Lys Ser Tyr Gln Cys Leu Lys Glu Thr Cys Glu Lys Leu
385 390 395 400

Gly Tyr Gln Trp Lys Lys Ser Cys Met Asn Gln Val Thr Ile Ser Thr
405 410 415

Thr Asp Arg Arg Asn Asn Lys Leu Ile Phe Lys Val Asn Leu Leu Glu
420 425 430

Met Asp Asp Lys Ile Leu Val Asp Phe Arg Leu Ser Lys Gly Asp Gly
435 440 445

Leu Glu Phe Lys Arg His Phe Leu Lys Ile Lys Gly Lys Leu Ile Asp
450 455 460

Ile Val Ser Ser Gln Lys Val Trp Leu Pro Ala Thr
465 470 475

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 742 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
"Mouse Chk1"

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..742

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGA GAG TTG CCG TGG GAC CAG CCC AGT GAT AGC TGT CAG GAA TAT CTG
48
Gly Glu Leu Pro Trp Asp Gln Pro Ser Asp Ser Cys Gln Glu Tyr Leu
1 5 10 15

ATT GTA AAG AAA AAA AAA ACC TAT CTC AAT CCT TGG AAA AAA ATT GAT
96
Ile Val Lys Lys Lys Lys Thr Tyr Leu Asn Pro Trp Lys Lys Ile Asp
20 25 30

TCT GCT CCT CTG GCT TTG CTT CAT AAA ATT CTA GTT GAG ACT CCA TCA 144
Ser Ala Pro Leu Ala Leu Leu His Lys Ile Leu Val Glu Thr Pro Ser
35 40 45

TCA AGG ATC ACC ATC CCA GAC ATT AAG AAA GAT AGA TGG TAC AAC AAA 192
Ser Arg Ile Thr Ile Pro Asp Ile Lys Lys Asp Arg Trp Tyr Asn Lys
50 55 60

CCA CTT AAC AGA GGA GCA AAG AGG CCA CGC GCC ACA TCA GGT GGT ATG 240
Pro Leu Asn Arg Gly Ala Lys Arg Pro Arg Ala Thr Ser Gly Gly Met
65 70 75 80

TCA GAG TCT TCT AGT GGA TTC TCT AAG CAC ATT CAT TCC AAT TTG GAC 288
Ser Glu Ser Ser Ser Gly Phe Ser Lys His Ile His Ser Asn Leu Asp
85 90 95

TTT TCT CCA GTA AAT AAT GGT TCC AGT GAA GAA ACC GTG AAG TTC TCT 336
Phe Ser Pro Val Asn Asn Gly Ser Ser Glu Glu Thr Val Lys Phe Ser
100 105 110

AGT TCC CAG CCA GAG CCG AGA ACA GGG CTT TCC TTG TGG GAC ACT GGT 384
Ser Ser Gln Pro Glu Pro Arg Thr Gly Leu Ser Leu Trp Asp Thr Gly

115 120 125

CCC TCG AAC GTG GAC AAA CTG GTT CAG GGC ATC AGT TTT TCC CAG CCT 432
Pro Ser Asn Val Asp Lys Leu Val Gln Gly Ile Ser Phe Ser Gln Pro
130 135 140

ACG TGT CCT GAG CAT ATG CTT GTA AAC AGT CAG TTA CTC GGT ACC CCT 480
Thr Cys Pro Glu His Met Leu Val Asn Ser Gln Leu Leu Gly Thr Pro
145 150 155 160

GGA TCT TCA CAG AAC CCC TGG CAG CGC TTG GTC AAA AGA ATG ACG AGG 528
Gly Ser Ser Gln Asn Pro Trp Gln Arg Leu Val Lys Arg Met Thr Arg
165 170 175

TTC TTT ACT AAA TTG GAT GCG GAC AAG TCT TAC CAA TGC CTG AAA GAG 576
Phe Phe Thr Lys Leu Asp Ala Asp Lys Ser Tyr Gln Cys Leu Lys Glu
180 185 190

ACC TTC GAG AAG TTG GGC TAT CAG TGG AAG AAG AGT TGT ATG AAT CAG 624
Thr Phe Glu Lys Leu Gly Tyr Gln Trp Lys Lys Ser Cys Met Asn Gln
195 200 205

GTT ACT GTA TCA ACA ACT GAT AGA AGA AAC AAT AAG TTG ATT TTC AAA 672
Val Thr Val Ser Thr Thr Asp Arg Arg Asn Asn Lys Leu Ile Phe Lys
210 215 220

ATA AAT TTG GTG GAA ATG GAT GAG AAG ATA CTG GTT GAC TTC CGA CTT 720
Ile Asn Leu Val Glu Met Asp Glu Lys Ile Leu Val Asp Phe Arg Leu
225 230 235 240

TCT AAA GGC GAC GGC TAC AAT T 742
Ser Lys Gly Asp Gly Tyr Asn
245

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 247 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Gly Glu Leu Pro Trp Asp Gln Pro Ser Asp Ser Cys Gln Glu Tyr Leu
1 5 10 15

Ile Val Lys Lys Lys Lys Thr Tyr Leu Asn Pro Trp Lys Lys Ile Asp
20 25 30

Ser Ala Pro Leu Ala Leu Leu His Lys Ile Leu Val Glu Thr Pro Ser
35 40 45

Ser Arg Ile Thr Ile Pro Asp Ile Lys Lys Asp Arg Trp Tyr Asn Lys
50 55 60

Pro Leu Asn Arg Gly Ala Lys Arg Pro Arg Ala Thr Ser Gly Gly Met
65 70 75 80

Ser Glu Ser Ser Ser Gly Phe Ser Lys His Ile His Ser Asn Leu Asp
85 90 95

Phe Ser Pro Val Asn Asn Gly Ser Ser Glu Glu Thr Val Lys Phe Ser
100 105 110

Ser Ser Gln Pro Glu Pro Arg Thr Gly Leu Ser Leu Trp Asp Thr Gly
115 120 125

Pro Ser Asn Val Asp Lys Leu Val Gln Gly Ile Ser Phe Ser Gln Pro
130 135 140

Thr Cys Pro Glu His Met Leu Val Asn Ser Gln Leu Leu Gly Thr Pro
145 150 155 160

Gly Ser Ser Gln Asn Pro Trp Gln Arg Leu Val Lys Arg Met Thr Arg
165 170 175

Phe Phe Thr Lys Leu Asp Ala Asp Lys Ser Tyr Gln Cys Leu Lys Glu
180 185 190

Thr Phe Glu Lys Leu Gly Tyr Gln Trp Lys Lys Ser Cys Met Asn Gln
195 200 205

Val Thr Val Ser Thr Thr Asp Arg Arg Asn Asn Lys Leu Ile Phe Lys
210 215 220

Ile Asn Leu Val Glu Met Asp Glu Lys Ile Leu Val Asp Phe Arg Leu
225 230 235 240

Ser Lys Gly Asp Gly Tyr Asn
245

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ACGTGGACAA ACTGGTTCAG G 21

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CTGATAGCCC AACTTCTCGA A 21

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GGCTCTGGGG AATCCTGGTG AATATAGTGC TGC 33

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TCCCCTGAAA CTTGGTTTCC ACCAGATGAG 30

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GTTGAGACTC CATCATCAAG G 21

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TCTGGCTGGG AACTAGAGAA C 21

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 98/18558

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/54 C12N15/70 C12N9/12 C12N1/21 C12Q1/48
C07K16/40

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C12Q C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HILLIER L. ET AL.: "The WashU-Merck EST project, AC AA224307" EMBL DATABASE,22 February 1997, XP002087721 Heidelberg see the whole document ---	1,3-7,9, 11-14
X	MARRA M. ET AL.: "The WashU-HHMI Mouse EST project, AC AA122952" EMBL DATABASE,22 November 1996, XP002087722 Heidelberg see the whole document ---	2-6,8,9, 11-14

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 December 1998	29/12/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl. Fax: (+31-70) 340-3016	Kania, T

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	AL-KHODAIRY F ET AL: "Identification and characterization of new elements involved in checkpoint and feedback controls in fission yeast." MOLECULAR BIOLOGY OF THE CELL, (1994 FEB) 5 (2) 147-60. JOURNAL CODE: BAU. ISSN: 1059-1524., XP002087724 cited in the application see the whole document	1-22
A	WALWORTH N. AND BERNARDS R.: "rad-dependent response of the chk1 -encoded protein kinase at the DNA damage checkpoint 'see comments!." SCIENCE, vol. 271, 19 January 1996, pages 353-356, XP002087725 see the whole document	1-22
A	CARR A M: "Checkpoints take the next step 'comment!." SCIENCE, (1996 JAN 19) 271 (5247) 314-5. JOURNAL CODE: UJ7. ISSN: 0036-8075., XP002087726 see the whole document	1-22
A	WO 97 09433 A (MEDICAL RES COUNCIL ;CARR ANTONY MICHAEL (GB)) 13 March 1997 see the whole document, esp. p. 32	1-22
A	WO 97 18323 A (ICOS CORP ;HOEKSTRA MERL F (US); HOLTZMAN DOUG A (US); KEEGAN KATH) 22 May 1997 see the whole document	1-22
P,X	SANCHEZ Y ET AL: "Conservation of the Chk1 checkpoint pathway in mammals: linkage of DNA damage to Cdk regulation through Cdc25 'see comments!." SCIENCE, (1997 SEP 5) 277 (5331) 1497-501. JOURNAL CODE: UJ7. ISSN: 0036-8075., XP002087723 see the whole document	1-22
P,X	FLAGGS G. ET AL.: "Atm-dependent interactions of a mammalian chk1 homolog with meiotic chromosomes." CURRENT BIOLOGY, (1997 DEC 1) 7 (12) 977-86. JOURNAL CODE: B44. ISSN: 0960-9822., XP002087728 see the whole document	1-22

-/--

1

INTERNATIONAL SEARCH REPORT

Inte ›nal Application No
PCT/US 98/18558

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropnate, of the relevant passages	Relevant to claim No.
T	WEINERT T: "A DNA damage checkpoint meets the cell cycle engine 'comment!." SCIENCE, (1997 SEP 5) 277 (5331) 1450-1. JOURNAL CODE: UJ7. ISSN: 0036-8075., XP002087727 see the whole document	1-22

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte onal Application No
PCT/US 98/18558

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 9709433 A	13-03-1997	AU	6884696 A	27-03-1997
		BR	9610168 A	11-08-1998
		CA	2231190 A	13-03-1997
		EP	0856058 A	05-08-1998
		NO	980957 A	05-05-1998
		PL	325427 A	20-07-1998
WO 9718323 A	22-05-1997	AU	1461197 A	05-06-1997
		CA	2210650 A	22-05-1997
		CZ	9702547 A	18-03-1998
		EP	0807169 A	19-11-1997
		FI	973005 A	15-09-1997
		NO	973279 A	16-09-1997
		PL	322876 A	02-03-1998
		SK	111597 A	06-05-1998